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14. ABSTRACT
Breast cancer cells are known to bear determinants that would allow tumor specific immune responses. However, initiation and amplification of such immune responses are critically dependent upon the balance in TH1 and TH2 cytokine profiles. This molecular epidemiological study evaluates the impact that variability in cytokine profiles, (inferred from functional polymorphisms in cytokine genes), may have on breast cancer risk among urban African-American women. In the first phase of the study, DNA collected and approved for additional study as part of a previously funded Case-Control investigation (n=1600) will be assessed for cytokine polymorphisms. Because cytokine profiles are also known to be affected by environmental factors, particularly levels of stress, this study also evaluates the relative contribution of genotype and stress influences using data collected for that purpose from a sub-sample of healthy Controls (n=400) recruited from the "graduates" of the larger study. Results will allow evaluation of the possibility that deficits in cytokine responses due to genetic or environmental factors may contribute to breast cancer risk. Based on these findings, women at risk for breast cancer because of polymorphisms in genes important to effective immune surveillance could be targeted for innovative prevention strategies including stress reduction and immune modulators.

15. SUBJECT TERMS
Immune surveillance, cytokines, psychoneuroimmunology

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Table of Contents

Immune surveillance, cytokines and breast cancer risk:
Genetic and psychological influences in African American women

Principal Investigator: Dr. Dana H. Bovbjerg

INTRODUCTION:

This project was grounded in the theoretical perspective of immune surveillance. Although the importance of immune surveillance in women's risk of developing breast cancer has yet to be directly tested, perhaps reflecting the practical difficulties of research on this topic. Traditional case-control approaches are problematic for at least two reasons: 1) because cancer treatment (e.g., surgery, chemotherapy) affects immune measures and thus confounds interpretation of differences; 2) even if functional immune assessments can be completed prior to treatment, any differences seen between cases and controls could be due to effects of the cancer on immune function rather than to a failure of immune surveillance to eliminate transformed cells, which we know would have had to have taken place years before the tumor reached a detectable size. These concerns could in principle be addressed with a truly prospective cohort study. Immune surveillance functions could be assessed in blood samples collected from a large sample of currently healthy women. The development of breast cancer in the sample could then be tracked over ensuing years until sufficient numbers of women developed clinical evidence of disease to have the statistical power to examine risk associated with the prior assessment of immune surveillance mechanisms. Given the relatively low risk of any woman's developing breast cancer in any given year and the increasing risk with age, such a study would require a very large sample and a decade or two of follow-up time.

An alternative research strategy that avoids these methodological problems has been initiated in our ongoing project, which takes a molecular epidemiological approach. We reasoned that effective immune surveillance mechanisms will be critically dependent upon normal cytokine responses to challenge and that dysregulation of cytokine responses may increase the risk of breast cancer. More specifically we hypothesized that women whose cytokine responses tend to favor humoral (Type 2) over cell-mediated (Type 1) responses may be at risk for developing breast cancer. This balance is in part determined by an individual's genotype, as demonstrated by functional associations between common polymorphisms in cytokine genes and assessments of cytokine responses in vitro and in vivo. Thus, as has been done extensively in the infectious disease literature, a case control study design including assessments of cytokine polymorphisms can be used to explore possible contribution of differences in cytokine responses to the risk of breast cancer. It should be noted that any associations found between the frequencies of cytokine polymorphisms and breast cancer risk cannot be directly attributed to differences in immune surveillance, as it is increasingly recognized that heightened pro-inflammatory processes triggered by cytokine responses can also contribute to increased cancer risk.

Since the time that this study was initiated, there have been three developments in the literature that make the ongoing study both more interesting and more complex. First,

several groups have reported finding differences between African American (AA) women and European American (EA) women in the frequency of specific polymorphisms in cytokine genes. For example, since the early report by Hoffman et al. (2002) describing significant differences between AA and EA in the frequencies of polymorphisms in IL-2, IL-6, and IL-10, there have been a number of other reports of such differences from other laboratories. For example, Hassan et al. (2003) reported differences between AA and EA in frequencies for IL-6 and IFN-gamma polymorphisms. Ness et al. (2004) reported frequency differences for IL-1, IL-6, IL-10 polymorphisms. Menon et al (2006) found frequency differences for TNF-alpha and TNF receptor subtypes. Velez et al. (2007) reported differences in frequencies of IL-6 and IL-6 receptor polymorphisms. Second, there have been several reports, largely from EA samples, of differences between breast cancer patients and controls in the frequency of specific polymorphisms in cytokine genes (e.g., Azmy et al., 2004; Hefler et al, 2005; Gaudet et al., 2007). Third, there has recently begun to be an appreciation of possible interactions between cytokine genotypes and other possible risk factors for breast cancer (Slattery et al. 2007).

Little is currently known about such effects in African American women. In the context of a previously funded case-control study (n=1600), we are evaluating the role of polymorphisms in cytokine genes associated with dysregulation in relation to breast cancer risk. In a sub-sample of healthy control subjects we are also exploring the relative contribution of genotype (cytokine polymorphisms) and environmental influences (e.g., stress-induced immune modulation) to cytokine responses.

The study is linked to two similar projects (Ambrosone, PI), one approved for funding as part of a Behavioral Center of Excellence award from the Army (DAMD-17-01-1-0334, Bovbjerg, PI) and the other funded by and R01 from the NCI. These “parent” projects draw on collaborations with physicians at NYC hospitals with large referral patterns for AA to recruit newly diagnosed breast cancer patients, as well as collaborations with the Department of Health in New Jersey. Age-matched controls are selected using Random Digit Dialing (RDD). Patients consenting to participate undergo an interview and provide a blood specimen for DNA extraction. For our piggy-backed study, appropriate banked DNA can be genotyped for the cytokine polymorphisms of interest. Additional newly obtained blood specimens from consenting Control participants are processed for cytokine responses (phenotype), and an additional set of questionnaires focused on psychological stress is completed at the time of the blood draw. Analyses will be conducted using standard approaches when appropriate sample sizes are reached.

This study synthesizes concepts from behavioral research and molecular epidemiology to address critical questions regarding breast cancer etiology. By exploring hypotheses related to psychoneuroimmunology and using technology and paradigms from molecular epidemiology, this research may make important contributions to identifying causes of breast cancer so that it may be eradicated. By examining case-control differences in cytokine polymorphisms, the role of this aspect of immune function in breast cancer may be elucidated. Furthermore, the evaluation of stress effects on cytokine responses in vitro, particularly in relation to genotype, may provide compelling

support for a possible role of stress in breast cancer etiology.

BODY:

Statement of Work

Task 0: Successful application for HSRRB approval though USAMRAA office
Task 1: Setting up study procedures
Task 2: Inclusion of 1600 Case and Control participants for genotyping
Task 3: Inclusion of 400 Control participants for phenotyping
Task 4: Cytokine evaluation of frozen stimulated samples
Task 5: Analysis of acquired cellular event flow cytometry data
Task 6: Statistical analysis of cytokine genotype data and preparation of manuscripts
Task 7: Statistical analysis of cytokine phenotype data and preparation of manuscripts

As previously reported, we have completed Tasks 0 and 1. HSRRB approval was granted in November 2004. Although the recruitment of Case Control participants to the parent studies has been slower than anticipated, we have made considerable progress on Task 2. We now have access to DNA from more than 1000 Case and Control participants that can be batch genotyped for the cytokine polymorphisms of interest. We have established procedures for coordination with the Molecular, Diagnostic and Research Core of the "parent" Behavioral Center of Excellence (Bovbjerg, PI) as well as with the Ambrosone laboratory involved in the parent R01. We have also been actively contacting Control participants for possible inclusion in the phenotyping portion of this study, but have fallen well behind anticipated enrollment rates. We have now completed 58 interviews.

There are two primary reasons that enrollment has been slower than anticipated. First, the recruitment of Control participants to the parent studies has been slower than anticipated. Second, we have found that we over-estimated the proportion of Control participants from the parent studies who are eligible, and available, for participation in the phenotyping study. Addressing the first problem is beyond the scope of this project as it has to do with the two parent projects from which we are drawing DNA samples. Those projects have recently (September 1, 2007) been reorganized with recruitment put under the direction of a faculty member with more than 20 years experience with directing large scale human studies, who has focused resources on the most productive sites for recruitment. The pace of recruitment has substantially increased since this reorganization. Addressing the second problem is difficult because the primary reason that Control participants have not been eligible for the phenotyping study is that these women have taken medication in the previous month. While this exclusion criterion eliminates the potential for confounding effects of medication on cytokine production, it has severely limited the number of eligible subjects, making it not only difficult to recruit the anticipated numbers, but also raising issues of generalizability. We therefore propose to follow the tradition in the cytokine phenotyping literature and eliminate this exclusion criterion. Instead we will explore possible effects of medication through

statistical evaluation of the resulting data. In addition, we propose to extend the age range of eligible subjects beyond 64 to be consistent with changes in the parent studies from which our sample is drawn. Both these changes must await final MSSM IRB and HSRRB approval before being implemented.

In July 2007, we received a no-cost extension to the grant period. While we anticipate improved rates of recruitment to the phenotyping portion of the study, we are unlikely to reach the target of 400 subjects. When we reach 80 participants, we therefore plan to conduct preliminary analyses that will allow us to determine effect sizes for hypothesized relationships between psychological variables and cytokine production. Based on those effect sizes, we will readdress statistical power considerations and on that basis may request modification of the statement of work to reduce the final sample size.

KEY RESEARCH ACOMPLISHMENTS:

Task 2: 1078 Case and Control participants included for genotyping study
Task 3: 58 interviews completed and samples frozen for batch analyses

REPORTABLE OUTCOMES:

At this point in the research, no reportable outcomes are yet available.

CONCLUSIONS:

If the results of the proposed research are consistent with study hypotheses, the study could have profound implications for the eradication of breast cancer. The results of the proposed research may suggest new means of evaluating genetic risk of breast cancer in healthy women, as well as novel intervention strategies for long term reduction of that risk, including stress reduction, as well as biological response modifiers designed to ameliorate dysregulation of cytokine profiles.

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APPENDICES:
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